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Effects of Different Heating Treatment and Storage Time on Formation of Resistant Starch from Potato Starch

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ABSTRACT

Resistant starch (RS), a polysaccharide, shows physiological properties similar to dietary fiber, and could be produced by processing. In this research, potato starch was used as material to investigate the effects of different heating treatments (water bath, high pressure autoclave and water bath-high pressure autoclave) and storage time (at 7°C for 7 and 14 d) on the RS content (RSC). According to the results, the RSC of potato starch processed by different heating treatments was in the range 10.61-26.00%. The potato starch processed by high pressure autoclave had a higher RSC than the water bath heating treatment without storage, but was close to the RSC from a combination of the water bath-high pressure autoclave heating methods. After storage for 7 d, the RSC was found to have increased with the water bath heating method, whereas it decreased with the high pressure autoclave heating method. However, there was no significant difference in the RSC between the heating methods when storage time was extended from 7 to 14 d.

Keywords: potato starch, resistant starch, process, heating treatment, storage time

INTRODUCTION

Resistant starch (RS) is defined as the sum of the starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Goni *et al*., 1996). Many studies have reported that RS has physiological benefits resembling soluble dietary fiber. It presents an exciting new potential as a food ingredient, since it is thermally stable and survives most food processes (Koksel *et al*., 2008). Food containing RS may be useful in the control of diabetes and obesity, by reducing the increase in the blood glucose level after a meal (Anderson and Guraya, 2006).

RS has been classified into four types: physically inaccessible starch (RS I), resistant starch granules (RS II), retrograded starch (RS III) and chemically modified starch (RS IV) (Garcia-Alonso *et al*., 1999; Shamai *et al*., 2003; Walter *et al*., 2005; Onyango *et al*., 2006; Mun and Shin, 2006; Murphy *et al*., 2008; Ozturk *et al*., 2009). RS may be found in both unprocessed foods, such as raw potatoes and bananas, and in processed foods (Kumari *et al*., 2007). In most cases, food processing involving heat and moisture will

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destroy RS I and II, but forms RS III (Kim *et al*., 2006). RS III formed during processing is associated with amylose retrogradation, so therefore, it could be influenced by factors, including amylose content, chain length, autoclaving temperature, storage time and temperature (Ozturk *et al*., 2009). Additionally, processed foods invariably undergo storage at moderate and low temperature before consumption. Storage and processing techniques may affect both the gelatinization and retrogradation processes (Garcia-Alonso *et al*., 1999; Noda *et al*., 2008), which are critical in determining the quantity of RS III formed.

Many materials and methods for RS III formation have been investigated, but most of them have the disadvantages of low yield and high cost. Thus, investigation is important to identify ways to increase the RS III content using cheap materials and to develop a low cost method for improving the functional properties of foods.

Potato starch is widely applied in the food industry for the manufacture of food products, due to its high swelling power and viscosity (Zaidul *et al*., 2008). In addition, potato starch contributes to the textural properties of many foods and is widely used in food and industrial applications as a thickener, gelling agent, bulking agent and water holding agent (Singh *et al*., 2006). Potato starch is a useful and cheap starch material in food processing. In particular, the large granule size, typical B-crystalline structure and high concentration of covalently bound phosphate result in the digestibility of potato starch being extremely low (Noda *et al*., 2008). Although many properties of potato starch have been studied, relatively little work has been reported on the effects of different heating treatments and parameters on the formation of RS III of potato starch. Therefore, the current research was conducted to investigate and compare the effects of different heating treatments (water bath, high pressure autoclave and water bath-high pressure autoclave) and storage time (at 7°C for 7 and 14 d) on the RS content (RSC) of potato starch with low cost.

MATERIALS AND METHODS

Materials

Potato starch (Avebe, Netherlands), containing less than 0.5% ash, was purchased from Wang Laichang Enterprise Co. Ltd. (Kaohsiung, Taiwan) and stored in a refrigerator with a practical temperature of 7°C before being used in the different processing experiments. The chemicals, including sodium phosphate monohydrate and sodium phosphate dibasic, were purchased from Mallinckrodt Baker Inc (Phillipsburg, USA). The α-amylase (Sigma A-3176), amyloglucosidase (Sigma A-7095), and protease (Sigma P-2143) were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Different heating treatments

Water bath treatment

The experiment with the water bath treatment was carried out using a slight modification of the method described by Garcia-Alonso *et al*. (1999) and Fredriksson *et al*. (2000). For each treatment, potato starch (80 g on a dry basis) was weighed in a 500-mL beaker before 400 mL distilled water was added. The beaker was placed on a laboratory stirring/hot plate and mechanically stirred by a stirring bar (40 × 8 mm) at ambient temperature for 30 min, then covered with aluminum foil and heated in a water bath tank (SB-7D, Hipoint, Taiwan), with the temperature set at 90°C, for 30, 60 or 90 min. After treatment, the starch paste was cooled at ambient temperature for 2 h.

High pressure autoclave treatment

The high pressure autoclave treatment was performed using the method of Shamai *et al*. (2003) and Huth *et al*. (2000), with a slight modification. For each treatment, potato starch

(180 g on a dry basis) was weighed in a 1,000-mL flask before 720 mL distilled water was added (starch:water = 1:4, w/v). The flask was placed on a laboratory stirring/hot plate and mechanically stirred by a stirring bar at ambient temperature for 30 min, then covered with aluminum foil and heated by a speedy autoclave (TM-328, Tomin medical equipment Co., Ltd., Taiwan) at 121°C for 1, 2, or 3 h at a pressure of 15 psi. After each treatment, the starch gel was cooled at ambient temperature for 2 h.

Water bath-high pressure autoclave treatment

The most extreme RSC conditions from the water bath and high pressure autoclave treatments and a combination of the above two methods were used for the water bath-high pressure autoclave method, which was also slightly modified using a previously described method of Koksel (2008). For each treatment, potato starch solution (50 g 500 mL^{-1}) was weighed in a 1,000-mL beaker and the beaker subsequently was placed on a laboratory stirring/hot plate and mechanically stirred by a stirring bar (40 mm × 8 mm) at ambient temperature for 30 min, then covered with aluminum foil and heated at 90°C in a water bath tank (SB-7D, Hipoint, Taiwan) for 60 min. Then, the sample was heated by a speedy autoclave (TM-328, Tomin medical equipment Co., Ltd., Taiwan) at 121°C for 1 h at a pressure of 15 psi. After treatment, the heated sample was cooled at ambient temperature for 2 h.

Sample preparation

The starch paste, gel and heated samples were divided into three batches. One of the three batches was directly dried at 60°C in a constant-temperature oven (SR-T7, Spain). The other two batches were placed in separate plastic bags and stored in a refrigerator with a practical temperature of 7°C for 7 and 14 d, respectively. After storage, these samples were dried at 60°C in a constant-temperature oven. The dried samples were pulverized by a laboratory grinder, using ASTM 80 and 100 mesh sieves to select a suitable particle size (150-180 μm) for further RSC analysis.

Resistant starch content

The RSC analysis was carried out using the method of Onyango *et al*. (2006), with a slight modification. Each treated potato starch sample (0.4 g on a dry basis) was weighed in a pre-weighed centrifuge tube, then 20 mL phosphate buffer (pH 6.0, 55.6 mM) and 0.16 g α-amylase (Sigma A-3176) were added and incubated at 37°C for 16 h. The sample was cooled to ambient temperature before adjusting to pH 4.5 using phosphoric acid solution (2 mL/100 mL). Then, 0.4 mL amyloglucosidase (Sigma A-7095) was added and the sample was incubated at 60°C for 30 min. After digestion, the potato starch samples were centrifuged at 4,000×g for 15 min by a high-speed micro centrifuge (CF15R, Hitachi, Japan) and the residue re-suspended in 20 mL phosphate buffer (pH 7.5, 0.08 M). Then, 0.4 mL protease (Sigma P-2143) was added and incubated at 42°C for 4 h. The sample was centrifuged at 6,000×g for 15 min then dried at 60°C to constant weight in a constant-temperature oven and weighed to determine the RS. The analysis was performed in duplicate. The RSC was calculated using Equation 1:

$$\text{RSC (\% dry basis)} = (\text{resistant starch weight}/\text{sample weight}) \times 100\% \quad (1)$$

Statistical analysis

Analysis of variance of the data was performed using the SAS statistical software (Windows V9.1, SAS Institute Inc., Cary, NC, USA). Comparisons among mean values were processed by Duncan's multiple range test and the level of significance was defined at $P<0.05$.

RESULTS AND DISCUSSION

RSC of potato starch under water bath and high pressure autoclave treatments

The RSC of untreated potato starch was 79.09±2.28% (data not shown). However, this type of RSC in the raw potato is classified as RS II (Murphy *et al*., 2008), which is destroyed during food processing (Faraj *et al*., 2004; Kim *et al*., 2006). Hence, the current research focused on RS III, which is a thermally stable enzyme-resistant starch (Chung *et al*., 2003).

The RSC of potato starch under the water bath and high pressure autoclave treatments is shown in Table 1. The RSC range was 10.61-26.00% and was mainly associated with the amylose content. Walter *et al*. (2005) also reported a positive correlation between the RSC and amylose content, due to amylose having a high degree of polymerization and forming enzyme-resistant double helices stabilized by hydrogen bonds. Amylose is the principle starch fraction required for the formation of RS III (Onyango *et al*., 2006).

The results of the RSC analysis of potato starch from the water bath and high pressure autoclave treatments showed that potato starch treated for 1 h by the above two methods produced the highest RSC, suggesting that treatment for 1 h was more efficient in promoting RS formation, compared to the other periods. The RSC declined significantly (P<0.05) when the treatment time increased, due to the effects of continuing thermal hydrolysis of the starch (Onyango *et al*., 2006).

Furthermore, the high pressure autoclave treatment produced higher RSC values than the water bath treatment, and the high pressure autoclave treatment for 1 h had the highest RSC, since the structure modifications and/or rupture of starch molecules could occur and these fragments could combine with other molecules, creating new compounds resistant to enzymatic digestion (Walter *et al*., 2005). In addition, the water bath treatment was similar to boiling. Anderson and Guraya (2006) reported that while boiling starch had only a minimal effect on digestibility, autoclaving produced significant differences in digestibility between starch types. The results indicated that 1 h of high pressure autoclave treatment of potato starch was suitable for RS formation.

Table 1 Resistant starch content of potato starch after water bath and high pressure autoclave treatments.

Method	TT	RSC (%)
	30 min	10.61^{d}
WBT	60 min	14.05^{c}
	90 min	10.86^{d}
	1 hr	26.00^{a}
HPA	2 hr	20.78^{b}
	3 hr	23.16^{b}

WBT=water bath treatment
HPA=high pressure autoclave treatment
RSC=resistant starch content
TT=treatment time
$^{a-d}$ = Means with different superscript letters are significantly different (P<0.05).

Garcia-Alonso *et al*. (1999) indicated that the higher RSC yields in potato starch are due to the crystallinity of potato starch, which is different from cereal starch. Potato starch has been shown to be more resistant to enzymatic hydrolysis than other starches. Moreover, potato starch granules are bigger than cereal granules, and yet potato amylose chains have a much higher degree of polymerisation (DP) than cereal chains, which makes it easy to form enzyme-resistant double helices (Onyango *et al*., 2006).

RSC of potato starch under water bath, high pressure autoclave treatments and storage time

The analysis of variance of the potato starch content in the water bath treatment for different storage times is given in Table 2. It is clear from the results that TT, ST and TT*ST significantly (P<0.05) affected the RSC of potato starch during storage. The RSC values of potato starch after the different water bath treatments and storage times (7 and 14 d) are shown in Table 3. Compared to the results without storage (Table 1), the RSC of all samples increased after storage, as expected, due to the starch treated by heat in excess water allowing amylose retrogradation during storage that induced the formation of RS III (Chung *et al*., 2003). This indicated that the RSC would be increased by storage. Chou and Lin (2007) also reported starch exhibited a loss of order within the granule and swelling after processing due to gelatinization, and reordering during storage due to retrogradation, leading to an increase in the RSC. In addition, the RSC decreased when the treatment time was extended to 90 min, then stored for 7 and 14 d, with some of the decreases being significant (P<0.05). The decrease could be attributed to amylose being leached out of the intact starch granule, leading to a change in the proportion of amylose and amylopectin, causing a decrease in the RSC (Sagum and Arcot, 2000) during the water bath treatment. This is evident from the results shown in Table 3.

The results from the statistical analysis showed that TT, ST and TT*ST insignificantly (P>0.05) affected the RSC of the potato starch prepared using the high pressure autoclave treatment after storage for 7 and 14 d. The RSC values of the potato starch under different high pressure autoclave treatments and storage times are shown in Table 4. The RSC of all samples decreased after storage at 7 and 14 d, when compared with the results without storage (Table 1); this could be attributed to amylopectin retrogradation, which requires longer storage times of days or weeks (Garcia-Alonso *et al*., 1999). In addition, Tovar *et al*. (2002) reported that syneresis could be take place after starch gel storage for 24 h, due to amylopectin retrogradation. However, it might be not only the result of syneresis, but also due to the decrease in the RSC as a result of

Table 2 Results of the analysis of variance of resistant starch content of potato starch after different water bath treatments and storage times.

Source	DF	Anova sum of squares	Mean square	F Value	*P*>F
TT	2	45.959	22.979	45.37	<0.0001
ST	2	19.469	9.734	19.22	0.0006
TT*ST	4	10.742	2.685	5.30	0.0179

TT=treatment time; ST=storage time.

Table 3 Results of the resistant starch content of potato starch after the water bath treatment and different storage times.

ST (d)	TT (min)	RSC (%)
	30	16.24[a]
7	60	15.13[ab]
0	90	14.07[bc]
	30	15.76[a]
14	60	16.67[a]
	90	13.48[c]

TT=treatment time; ST=storage time; RSC=resistant starch content.

[a-c] = Means with different superscript letters are significantly different (P<0.05).

Table 4 Resistant starch content of potato starch after the high pressure autoclave treatment and different storage times.

ST (d)	TT (h)	RSC (%)
	1	17.54[ab]
7	2	14.90[b]
	3	18.28[a]
	1	14.95[b]
14	2	17.39[ab]
	3	16.94[ab]

TT=treatment time; ST=storage time; RSC=resistant starch content.

[a-b] = Means with different superscript letters are significantly different (P<0.05).

Table 5 Resistant starch content during storage after water bath and high pressure autoclave treatments.

ST (d)	Method	RSC (%)
7	WBH	15.15[a]
	HPA	15.30[a]
14	WBH	16.91[a]
	HPA	16.42[a]

ST=storage time; RSC=resistant starch content; WBT=water bath treatment; HPA=high pressure autoclave treatment;
[a] = Means with different superscript letters are significantly different (P<0.05).

Table 6 Effects of different storage times on resistant starch content of water bath-high pressure autoclave treated potato starch.

ST	RSC (%)
0	24.97[a]
7	22.01[ab]
14	18.95[b]

ST=storage time; RSC=resistant starch content.
[a-b] = Means with different superscript letters are significantly different (P<0.05).

prolonged storage, due to instability of the amylopectin crystals, particularly at refrigerated temperatures (Niba, 2003). Furthermore, the availability of water is essential in the formation of resistant starch, as it is involved in re-crystallization of amylose. In addition, the retrogradation of amylopectin is reversible, particularly with reheating (Niba, 2003). The starch gel and paste were dried at 60°C before the RSC analysis in this research. Garcia-Alonso *et al*. (1999) indicated amylopectin crystallites are less stable than those of amylose, with a melting point close to 60°C. High drying temperatures can result in the melting of retrograded amylopectin crystals that are unstable, and as mentioned before, in addition, the decrease in the RSC might be attributed to the drying treatment before RSC analysis.

The analysis of the average RSC from the water bath and high pressure autoclave treatments during storage (no storage = 0 d) is shown in Table 5. No statistically significant (P>0.05) differences in the RSC were observed using either the water bath or the high pressure autoclave treatment for any storage time. Garcia-Alonso *et al*. (1999) also found no differences in the degree of polymerization (DP) between the autoclaved and water-bath boiled samples, with an average chain length of 50-60 (Garcia-Alonso *et al*., 1999), suggesting that both gelatinization methods resulted in similar final products made from wheat, corn, rice or potato starch.

RSC of water bath-high pressure autoclave treated potato starch under different storage time

The RSC of potato starch from the water bath-high pressure treatment for different storage times was in the range 18.95-24.97% (Table 6). The RSC of potato starch was 24.97% after the water bath-high pressure autoclave treatment, and the decrease was insignificant (P>0.05) to 22.01% after storage for 7 d, but there was a significant (P<0.05) decrease to 18.95% when the storage time was extended to 14 d.

Compared with the results in Table 1, it is clear that a combination of the water bath and high pressure autoclave treatments produced higher RSC values than the water bath treatment alone, but the values were close to those from the high pressure autoclave treatment alone. Walter *et al*. (2005) reported that structural modifications and/or rupture of starch molecules can occur and these fragments can combine with other molecules during processing, creating new compounds that are resistant to enzymatic digestion. This action may explain the starch hydrolysis or disintegration that occurred during autoclaving with sufficient water, and then the recrystallization by association between linear amylose or between amylose and amylopectin through cooling (Onyango *et al*., 2006; Mun and Shin, 2006). The current data provided evidence that higher RSC values can be obtained by the high pressure autoclave treatment alone, removing the need for the double heating treatment, such as by a combination of the water bath and high pressure autoclave treatments.

Compared to the results of the RSC analysis without storage, the syneresis that took place during prolonged storage at a refrigerated temperature, due to the instability of the amylopectin crystals, resulted in a decrease in the RSC after storage. The availability of water is essential in the formation of resistant starch, as it is involved in the re-crystallization of amylose, causing a decrease in the RSC during the prolonged storage time used in the current research (Niba, 2003).

CONCLUSION

The current study applied different heating treatments and storage times to investigate the resistant starch content formation from potato starch. The results showed that different heating treatments and storage times may influence the resistant starch content of potato starch. The resistant starch content of potato starch treated by high pressure autoclave was higher than from using a water bath without subsequent storage. However, the resistant starch content of potato starch treated by water bath exhibited an increasing trend after storage, but the high pressure autoclave treatment did not. Analysis of the average resistant starch content, indicated that the water bath treatment and the high pressure autoclave treatment showed similar results during storage at 7°C for 7 and 14 d. The resistant starch content from the water bath-high pressure autoclave method was higher than from the water bath method, but similar to the results from the high pressure autoclave method, and decreased after storage at 7°C for 7 and 14 d.

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